New species for EU aquaculture

Deliverable Report

Deliverable No:	D17.5	**Delivery Month:**	56
Deliverable Title	Development of an industrial protocol for probiotic treatment of halibut larvae		
WP No:	11	**WP Lead beneficiary:**	P7. IMR
WP Title:	Larval husbandry – Atlantic halibut		
Task No:	17.3	**Task Lead beneficiary:**	P7. IMR
Task Title:	The effect of probiotics on larval microbiota and survival and development of an industrial protocol		
Other beneficiaries:	P22. SWH		
Status:	**Delivered**	**Expected month:**	48
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Objective: The objective of this deliverable was to develop a protocol for industrial use of probiotics in Atlantic halibut juvenile production.

Introduction

The commercial production of Atlantic halibut (*Hippoglossus hippoglossus*) fry is currently carried out in flow through systems (FT), while there is a growing consensus that a recirculation system (RAS) would offer more stable environmental and chemical water parameters that would lead to improved larval performance. The yolk sac and first feeding stages in halibut are performed in different rearing tanks. A RAS for both these stages are presented here. The first test of a RAS for the yolk sac stage was done in 2015 and a second, modified, was conducted in 2017. Both experiments were done without replicate silos. For the first feeding stage, experiments were conducted in 2016 and 2017. These experiments were done using triplicate tanks both for the RAS system and control tanks. This protocol is based on the second trial for both yolk sac and first feeding stages. A metagenomic analysis of the bacterial flora in the different systems is presented in Deliverable D17.3.

Yolk sac stage:

The yolk sac stage for halibut lasts for 43 days at 6 C°. Eggs are transferred to the silos approximately 3 days prior to hatch. At this time, a salinity gradient has been established in the upper part of the silo by use of freshwater. Hatching is synchronized by the use of light, which arrests hatching, and thereafter darkness to induce hatching. The salinity gradient is present during hatching and for one or two more days, depending of the buoyancy of the larvae. Recirculation is not used in this period. The silos used for water treatment and for larval rearing, are 5000 litres in volume (**Fig. 1**). Approximately 1 to 2 litres of eggs (40 000-80 000 eggs) are normally incubated in one silo, depending on the size of the egg batch. There is no feeding or any addition of organic material during this period and the larvae do not begin drinking activity before approximately day seven after hatching.

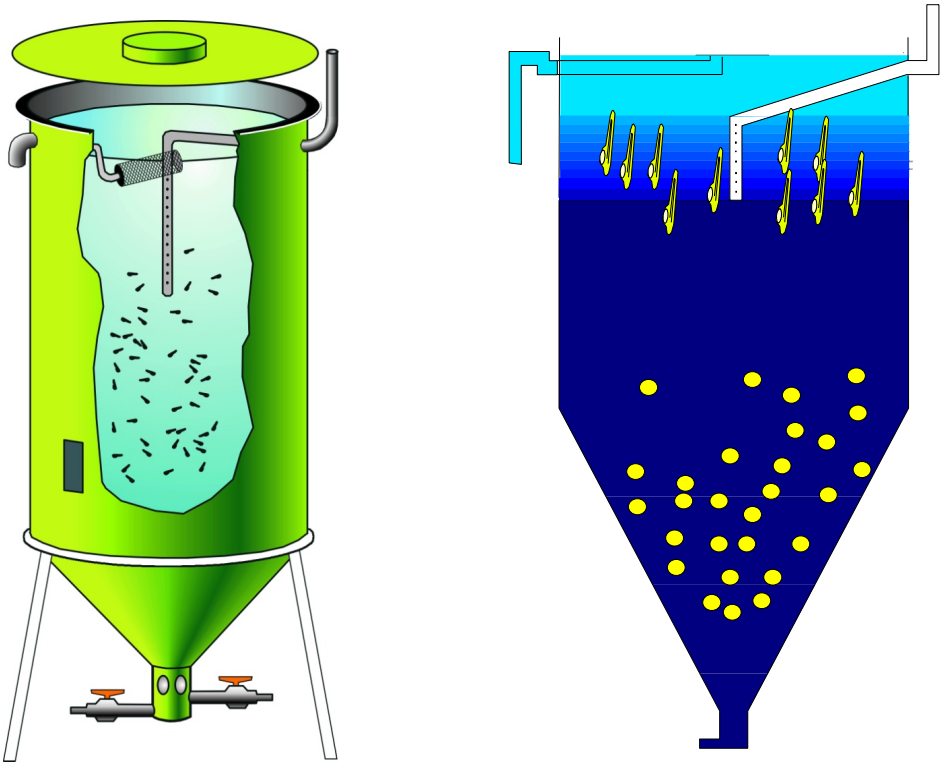

Figure 1. Schematic illustration of yolk sac silo.

Two trials were conducted before the protocol was finalized. In the first trial, water temperature was adjusted between the RAS silo (without larvae) and the silo inoculated with larvae (**Fig. 2**). In the second trial this temperature adjustment was done within the RAS silo, resulting in a more even temperature profile.

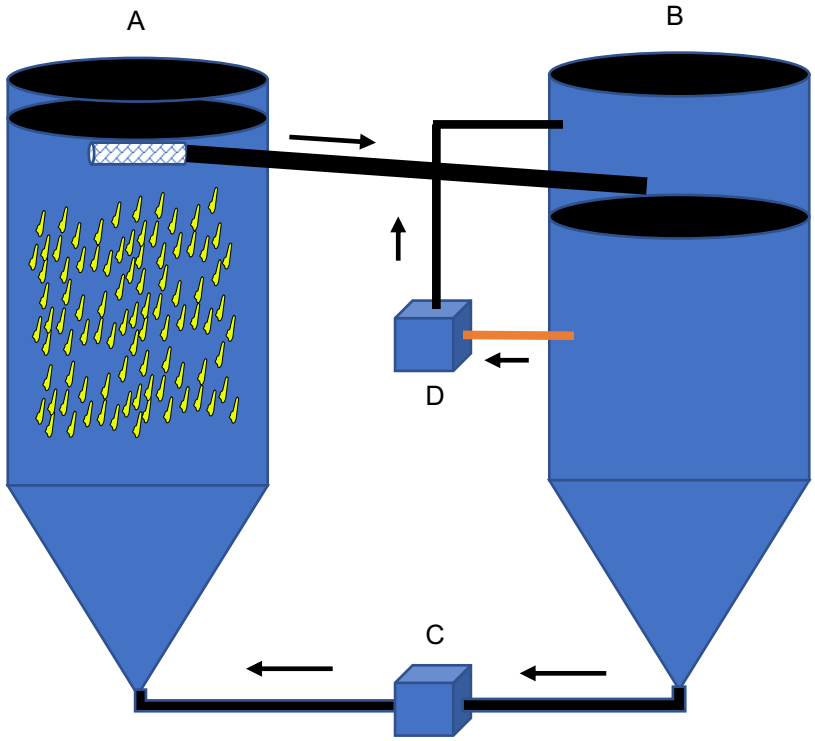

Figure 2. Illustration of the RAS used for yolk sac larvae. A= silo with larvae, B=water treatment, C= water pump including flowmeter. D= water cooler.

Protocol for Atlantic halibut yolk sac larvae:

Fill the yolk sac incubator and the RAS silo with seawater of full salinity.

Establish a salinity gradient in the larvae incubator approximately two days before egg incubation.

Maintain circulation of new seawater during hatching and until the larvae are distributed evenly in the incubator and the salinity gradient is removed. This can take one to 3 days depending on the buoyancy and distribution of the larvae.

Change the incoming water from FT to RAS.

Increase water flow from one $l \cdot min^{-1}$ at the start to a maximum of 5 $l \cdot min^{-1}$ within five days. Measure water temperature and the distribution of the larvae in the incubator daily. If the larvae are distributed in the bottom half of the incubator create a small turbulence by adding oxygen bobbles (ceramic diffusor) from the bottom of the silo.

Renew the layer of brackish water at the surface of the incubator (above the outlet sieve) once a week.
At approximately 260 day-degrees remove the layer of brackish water by lifting the outlet sieve. Illuminate the surface of the incubator and collect the larvae as they are coming to the surface with a 10-liter bucket and transfer them to start feeding units.

First feeding stage:

A RAS system from Tropical Marine Centre (TMC) has been used by the IMR for research on several cold-water and warm-water marine species (**Fig. 3**). In this set up, three first feeding tanks were connected to the same RAS system (**Fig. 4**).

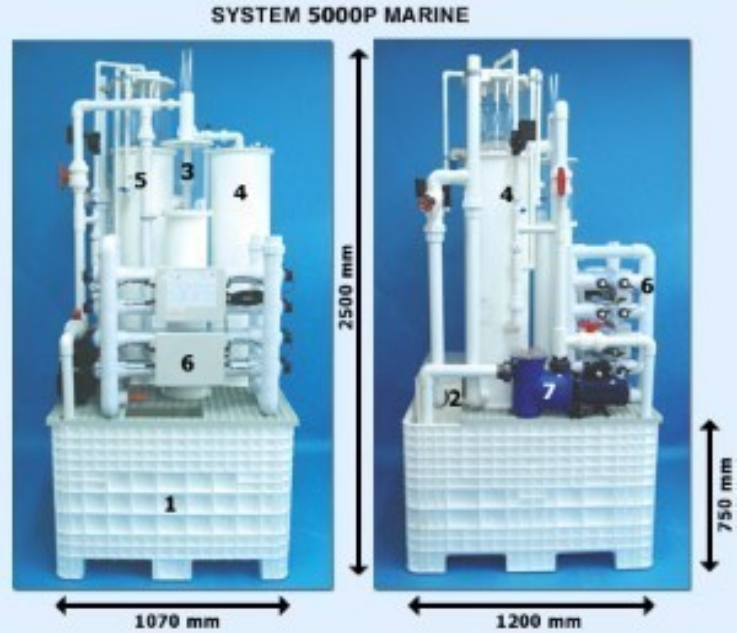

Figure 3. The RAS system P5000P MARINE from Tropical Marine Centre. The system consists of a reservoir (650 l), filter bags, sand filter, re-gassing / trickling biofilter and a protein skimmer.

Figure 4. First feeding tanks.

The first-feeding tanks were flat bottomed, with a volume of 1100 l and a water flow of 5 $l \cdot min^{-1}$. Water temperature was 12 ± 0.3°C during the whole period. The tanks had shadow frames to avoid illumination of

the walls and fluorescent (daylight) light source placed 70 cm above the water surface, giving a light intensity of approximately 400 lux at the surface. The tanks had central aeration near the bottom. The water outlet sieves were also in the center of the tanks, reaching from the bottom to the surface. Water inlets were placed near the tank wall approximately 10 cm below the surface. An automatic cleaning device fitted with car windshield wipers was mounted in each tank and was run once a day. After one rotation, a siphon was used to remove dead material. The water volume that was removed daily by siphoning represented the water exchange in the RAS. The recirculating volume was calculated to 97%. Water turbidity was created by use of dissolved clay (Sibelco, Vingerling K148, white) to an initial turbidity of 2 NTU. Approximately 10 g of clay was dissolved in one liter of freshwater and added to each tank twice a day. Before the water returned to the RAS unit it was filtered to remove *Artemia* and part of the clay (**Fig. 5**). The remaining clay was left in the RAS unit, mostly in the reservoir.

Figure 5. Bag filter and sedimentation tank. Water returned from the larvae tanks and entered the bag filter where large particles and *Artemia* were removed.

Protocol for feeding Atlantic halibut larvae under RAS condition:

Priming
Fill the RAS unit with seawater more than 30 days prior to larvae incubation. During this period the recirculation unit without tanks, a total of 650 l, must be conditioned by addition of a daily amount of 1.5 g NH_4Cl.

Preparation:
Measure NH_4 concentration and pH value once a week to see if the biofilter in the unit removes NH_4.

Keep water temperature stable at 12°C.

Fill the first feeding tanks with seawater the day before larvae incubation. Adjust aeration, waterflow and turbidity. Connect the tanks to the RAS unit.

Incubate approximately 5000 larvae per tank. Feed short-time enriched *Artemia* according to feeding protocol.

Daily routines:
Check and if necessary adjust water flow in protein skimmer (venturi pump), sand filter and biofilter in the RAS unit.

Exchange and clean bag filters prior to the RAS unit (excess *Artemia* and clay) and the bag filters in the RAS unit.

Refill sea water after the larvae tanks have been tended. Use a water level mark in the reservoir.

Weekly routines:
Measure NH_4 concentration and pH value.

Siphon clay from the bottom of the reservoir.

Other:
Feed and remove dead larvae according to larvae rearing protocol.

Deviations from Annex I and their impact:

Atlantic halibut differ from other marine species that spawn pelagic eggs, by having large eggs, a long lasting yolk sac stage (43 days at 6°C) and a very strict temperature requirement with low tolerance to temperatures above 6°C during yolk sac incubation. This made addition of probiotic bacteria to the rearing systems dificcult. The larvae start drinking activity approximately seven days after hatching, and it is likely that the intestine is colonized with bacteria during the yolk sac stage. Therefore, we did not introduce any exogenous probiotic bacteria either during the yolk sac stage or the first feeding stage, but instead used a long priming period to create a stable microbial environment in the rearing systems. As seen by the results reported in D17.2 this proved to be an efficient method to improve growth and survival.

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